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USE OF N-ACETYLCOLCHAMINE AS A MITOTIC POISON

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Use of N-acetylcolchamine as a Mitotic Poison

by

V. I. Khmelevskiy, F. Ya Zusman,
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13. ABSTRACT

The anti-mitotic activity of N-acetylcolchamine on Ehrlich ascites cancer cells has been studied in vivo in mice. It has been established that N-acetylcolchamine is an inhibitor of mitosis which, like colchicine, is able to block cell division at the stage of metaphase. Since N-acetylcolchamine is more readily available than colchicine, and is easily obtained in a chemically pure state by acetylation of colchamine, it is recommended for use in various biological studies in place of colchicine.

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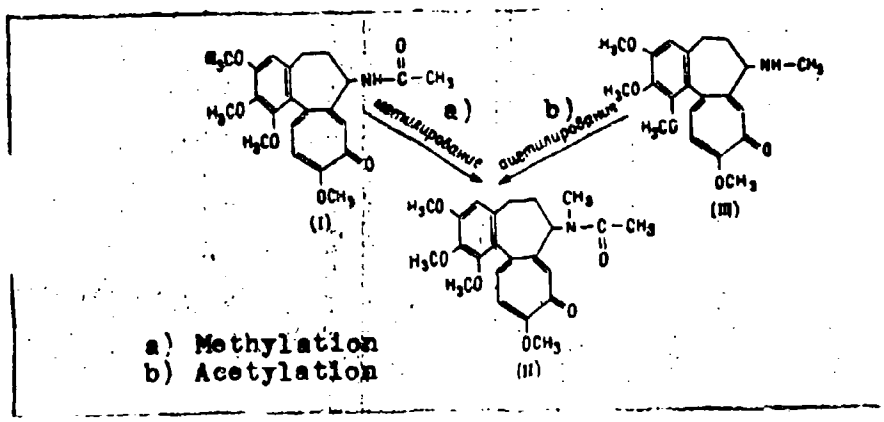
USE OF N-ACETYLCOLCHAMINE AS A MITOTIC POISON

N-acetylcolchamine was first obtained by Kiselev and Men'shikov (1953) by means of acetylation of colchamine with acetic anhydride. Later this substance appeared in the foreign literature (Ueno, 1953; Patent: Japan, 1956; Germany, 1959; Santavy, 1959; French Patent, 1962).

Proper attention was not paid at that time to detailed study of N-acetylcolchamine as an inhibitor of mitosis. There were data only on its toxicity, which is approximately equivalent to the toxicity of colcnicine (Filitis, 1957).



As can be seen from the structural formulas (I-III), N-acetylcolchamine (II) may be considered both as a derivative of colchamine (III), and of colchicine (I). Moreover

it is known that these alkaloids (I, III) differ markedly in their physiological effect (Sharapof, 1956). This comprehensive study of N-acetylcolchamine is of assured interest. The present work was devoted to a study of the effect of this substance on the division of Ehrlich ascites cancer cells.

Materials and Methods

Experiments were carried out in white non-pedigree mice (males 18-25 g), which were inoculated in the peritoneal cavity with 0.1 ml of Ehrlich ascites carcinoma fluid 6-7 days before injection of the preparation. N-acetylcolchamine was injected intraperitoneally in single doses of 1.5 and 10 Mg per mouse and 0.5 ml of physiologic solution at 9 a.m. For 15 hours after injection of the preparation, every three hours a portion of the animals of the experimental and control groups was sacrificed by decapitation, and smears were prepared from the ascites fluid of each animal, which were fixed in methanol, subjected to hydrolysis in 1 normal hydrochloric acid for 5 minutes and stained with azure-eosine. The number of cells per thousand in prophase and metaphase were calculated in the smears. Evaluation of the effect of the preparation was conducted according to the increase in mitotic index (MI), expressed in per cent (Meziya, 1963).

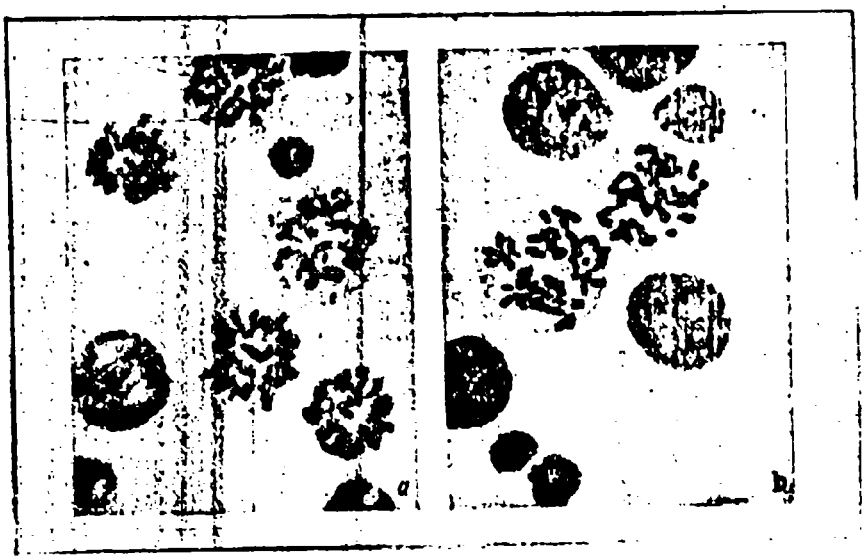
According to our observations, the physiologic solution which was used as a solvent, did not have a particular effect on the mitotic activity of the Ehrlich carcinoma cells. Thus, for example, three hours after injection of 0.5 ml of the indicated solution in the mice MI = 1.38 ± 0.37%. In the control animals during the same period after inoculation of tumor and at the same time of day this index was equal to 1.06 ± 0.3. This difference is statistically unreliable. Nevertheless, in experiments with N-acetylcolchamine animals were used for the control group which were injected once at 9 a.m. with physiologic solution at a dose of 0.5 ml per mouse. Statistical treatment of the data obtained was carried out by the method of comparison according to per cents (Genes, 1964).

Results and Discussion

The average values that the mitotic indices obtained in experimental and control groups are presented in the table.

Three hours after injection of the mice with 1 Mg of N-acetylcolchamine the MI increases to 2.13 ± 0.45% after nine hours, the number of dividing cells at the indicated dose is 4.45 ± 0.65%. The maximum value of the MI depends markedly on the injected dose of the substance. In our experiments it reached a value of 9.85 ± 0.94 (at a dose of 5 Mg) and 10.0 ± 0.95 (at 10 Mg). N-acetylcolchamine, like colchicine (Lettre, 1946; Alov, 1965), blocks mitosis at the stage of metaphase (Figure 1, a). At increased concentrations this substance disrupts the process of formation of mataphase platelets, and the chromosomes remain dispersed through the cytoplasm (Figure 1,b). In this case, moreover, a delay occurs in the entry of cells into mitosis, which is evident from the change in MI at doses of 5 and 10 Mg.

Thus, according to its effect on the process of mitosis, N-acetylcolchamine belongs to the typical representatives of the so-called colchicine-like substances, which cause K-mitosis. Considering the fact that N-acetylcolchamine is a more accessible substance than colchicine, and, moreover, in the process of acetylization of colchamine is obtained in the chemically pure state, it may be recommended for use in various biological studies as a preparation monotypic in effect with colchicine. GRAPHICS NOT REPRODUCIBLE



Effect of N-acetylcolchamine on Mitosis of Ehrlich Ascites Carcinoma Cells
a, at a dose of 1 Mg; b, at a dose of 10 Mg
Azure-eosine;

Conclusions

1. The antimytotic activity of N-acetylcolchamine on cells of Ehrlich ascites cancer was studied in vivo.

2. It was established that N-acetylcolchamine is an inhibitor of mitosis, which is able, like colchicine, to block cell division at the metaphase stage.

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Variation of mitotic indices (MI) in Ehrlich ascites carcinoma

Time after introduction of preparation in hours	N-acetylcolchamine									physiological solution	
	1 Mg			5 Mg			10 Mg				
	Number of animals	MI in %	Criterion of reliability	Number of animals	MI in %	Criterion of reliability	Number of animals	MI in %	Criterion of reliability	Number of animals	MI in %
3	10	2.13±0.45	—1.3	5	1.16±0.34	0.4	15	1.23±0.35	0.3	5	1.38±0.37
6	9	3.81±0.61	—1.9	5	0.46±0.21	3.6	19	0.95±0.30	2.5	5	2.34±0.48
9	8	4.45±0.65	—4.5	5	0.76±0.27	1	12	1.45±0.38	—0.5	5	1.18±0.34
12	10	3.31±0.56	—2.8	5	9.64±0.93	—8.2	24	5.75±0.74	—5.2	5	1.44±0.38
15	5	0.96±0.31	1.8	5	9.85±0.94	—7.6	14	10.0±0.95	—7.8	5	1.95±0.41

SUPPLEMENTARY

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